

Appl. No. 10/535,736  
Amendment dated April 12, 2010  
Reply to Office action of December 11, 2009

**Do not enter. /LJH/ 10 June 2010**

**Amendments to the Claims:**

This listing of claims will replace all prior versions, and listings, of claims in the application:

**Listing of Claims:**

Claim 1 (currently amended): A method for immobilizing biomolecules, which method comprises

contacting a solution containing a biomolecule or biomolecules provided with at least one tag with an immobilization substrate which has (i) binding sites for the biomolecule tag or tags, and (ii) activated reactive groups which form covalent bonds with the non-tag part of the biomolecule or biomolecules; and

immobilizing the biomolecule or biomolecules on the immobilization substrate through both (1) binding between the tag or tags and tag-binding sites of the immobilization substrate, and (2) forming covalent bonds between the reactive groups and the non-tag part of the biomolecule or biomolecules.

Claim 2 (previously presented): The method according to claim 1, comprising the steps of:

a first step wherein the reactive groups of the immobilization substrate which are capable of forming a covalent bond with the biomolecule or biomolecules to be immobilized are activated;

a second step wherein a solution containing the biomolecule or biomolecules to be immobilized is reacted with the immobilization substrate following the first step, and

wherein, in the second step, the biomolecule or biomolecules are immobilized on the immobilization substrate through interaction between the tag or tags and tag-binding sites of the immobilization substrate and covalent bonds formed between the reactive groups and the non-tag part of the biomolecule or biomolecules.

Claim 3 (original): The method according to claim 2, wherein the reactive groups are carboxyl groups, and in the second step, an amine coupling is formed between the carboxyl groups and an amino group on the biomolecule to be immobilized.

Claim 4 (previously presented): The method according to claim 2, wherein the tag is a histidine tag, and in the second step, an interaction is effected between the histidine tag and the immobilization substrate.

Claim 5 (original): The method according to claim 4, wherein, in the second step, an interaction is effected between the histidine tag and the immobilization substrate through a complex.

Claim 6 (original): The method according to claim 5, wherein, in the second step, an interaction is effected between the histidine tag and the immobilization substrate through a metal ion chelate.

Claim 7 (original): The method according to claim 6, wherein, in the second step, an interaction is effected between the histidine tag and the immobilization substrate through  $\text{Ni}^{2+}$  nitrilotriacetic acid (Ni-NTA).

Claim 8 (original): The method according to claim 6, wherein, in the second step, an interaction is effected between the histidine tag and the immobilization substrate through  $\text{Ni}^{2+}$  iminodiacetic acid (Ni-IDC).

Claim 9 (previously presented): The method according to claim 1, wherein the tag-binding site of the immobilization substrate is an antibody to the tag; further wherein the covalent binding is to a non-tag part of the biomolecules.

Claim 10 (original): The method according to claim 9, wherein the tag is a histidine tag, the antibody is an anti-histidine antibody and, in the second step, an interaction is effected between the histidine tag and the immobilization substrate through an anti-histidine antibody.

Claim 11 (previously presented): The method according to claim 1, wherein the tag is an inherent part of a native biomolecule.

Claim 12 (previously presented): The method according to claim 1, wherein the biomolecule is a protein.

Claim 13 (previously presented): A method for determining biomolecule-low molecular weight compound affinity and/or kinetics comprising:  
a step for reacting a sample containing a low molecular weight compound or compounds to be determined with an immobilization substrate to which a biomolecule or biomolecules have been immobilized using the method for immobilizing biomolecules as defined in claim 1, and  
a step for determining the affinity and/or kinetics of the low molecular weight compound or compounds contained in the sample for the biomolecule or biomolecules immobilized on the immobilization substrate.

Claim 14 (original): The method according to claim 13, wherein the affinity and/or kinetics of a biomolecule and a low molecular weight compound is determined using the principle of surface plasmon resonance (SPR) in the step for determining affinity and/or kinetics.

Claim 15 (previously presented): The method according to claim 13, wherein the biomolecule is a protein.

Claims 16-17 (cancelled)

Claim 18 (previously presented): An immobilization substrate comprising at least one immobilized biomolecule, wherein the biomolecule or biomolecules have been immobilized by the method defined in claim 1.

Claim 19 (previously presented): The immobilization substrate of claim 18, which comprises:

a substrate, and  
polysaccharide chains arranged on the substrate, into which are introduced reactive groups capable of forming covalent bonds with the non-tag part of a biomolecule or biomolecules to be immobilized thereon,  
wherein the biomolecule or biomolecules interact with the polysaccharide chain through a chelate and form covalent bonds with the reactive groups.

Claim 20 (previously presented): The immobilization substrate of claim 18, wherein the biomolecule is a protein.

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Claim 21 (previously presented): The method according to claim 12, wherein the protein is a recombinant protein produced with a tag.